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# Further Proof of the Cause and Infectiousness of Crown Gall

By CLAYTON O. SMITH

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# FURTHER PROOF OF THE CAUSE AND INFECTIOUSNESS OF CROWN GALL.

By CLAYTON O. SMITH.

The disease to be discussed in this bulletin is one of the most widespread and serious troubles known to plant life, for while other diseases may destroy a portion of the tree or crop, this trouble gradually weakens and frequently kills the tree affected. It is not the purpose of this paper to discuss the disease completely or in detail, but to present the results of some experimental work which has been done at the Southern California Pathological Laboratory upon its cause and power of infection.

## BRIEF HISTORY.

The crown gall or root-knot has been long known in Europe, the United States and Canada, and is probably world-wide in its distribution. Just when the first published account of it was printed we do not know, although it was probably fifty to seventy-five years ago. There is no uniformity of opinion among plant pathologists as to where this disease originated. Some authorities say it came from China or some country of Asia. From our own observations, however, it seems to be without question native to many of the soils of our country, and this appears to be especially true of California, since there are numerous instances of the occurrence of crown gall in nurseries on land where trees have never been grown before.

Crown gall was first extensively studied by J. W. Toumey,[1] who at that time was associated with the Arizona Experiment Station. He showed the pathogenic nature of the disease by inoculating soil with minced galls. He made cultures from the affected tissue and isolated a low form of plant known as a myxomycete or slime-mold. The number of his inoculations was not sufficient to be very conclusive, and subsequent work has shown the disease to be due to a species of bacterium.

In 1897 Cavara,[2] an Italian investigator, made cultures from knots of grapes, and succeeded in isolating a species of bacterium that produced knots from his artificial inoculations on two varieties of European grapes. His study and description of the organism, so far as they went, leave little doubt that he had found the true cause of the trouble.

In 1903, Dr. George G. Hedgcock,[3] while studying the knot of the grape, isolated a bacterial organism producing a white colony, but lost the cultures in transit. In 1904 he again isolated several kinds of organisms from grape galls; one of these was similar to the one of 1903 and produced knots when puncture inoculations were made in grapes.

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[1] Arizona Agricultural Experiment Station, Bulletin No. 33.
[2] Abstracts and Summary given in U. S. Department of Agriculture, Bureau of Plant Industry, Bulletin No. 213.
[3] U. S. Department of Agriculture, Bureau of Plant Industry, Bulletin No. 183.

Dr. E. F. Smith and Dr. C. O. Townsend, of the Bureau of Plant Industry, from 1904 to 1906 gave some attention to the study of gall on the Paris daisy, *Chrysanthemum frutescens,* and were successful in isolating a pathogenic bacterial organism with which they could produce the gall at will, if given certain favorable conditions of plant life. The organism was called by them *Bacterium tumefaciens.* Much subsequent work has been done by Dr. Smith and his associates, showing that this organism is capable of causing knots on a great number of different host plants.[1] This same organism has also been isolated from galls found on quite a number of different plants.

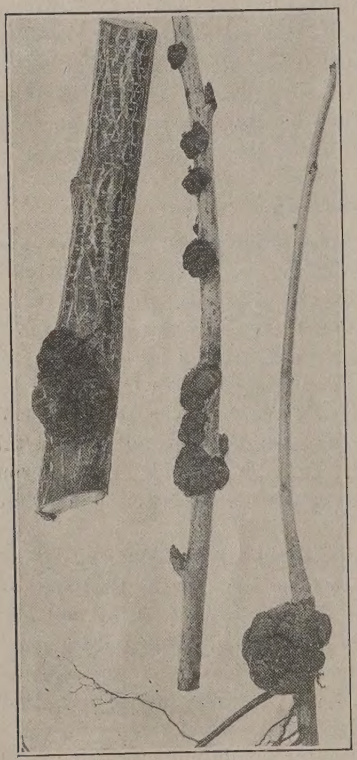

FIG. 1.—Artificial inoculations producing galls on *Prunus davidiana.* This tree, known sometimes as Chinese ornamental almond, has been recommended as a stock for stone fruits, but it promises no particular advantage with regard to the crown gall disease.

Plant pathologists have been very slow in attempting a thorough investigation of this disease until within recent years, and this may be readily accounted for because of the following reasons: (1) Professor Toumey published quite a complete paper on the subject, and this would naturally discourage an investigator from repeating his work. (2) The seeming hopelessness of finding a successful method of preventing the disease would cause workers to study first those diseases that could be more easily controlled. (3) The early workers in the field of plant pathology were mycologists rather than bacteriologists and would, from choice, study the fungous diseases. (4) In the diseased gall tissue, comparatively few bacteria are present and the microscope does not conclusively demonstrate the cause of the trouble under ordinary histological methods.[2]

[1] U. S. Department of Agriculture, Bureau of Plant Industry, Bulletin No. 213.

[2] In Bulletin 255, Bureau of Plant Industry, U. S. Department of Agriculture, special methods are described for demonstrating this organism in the cell.

## GENERAL DESCRIPTION OF THE DISEASE.

### Geographical Distribution.

The disease is common in every state and territory of the United States; it is found in Canada, South America, South Africa, the countries of Europe and Asia. In other words, the disease is nearly universal in its distribution and almost omnivorous as to its choice of a host.

### Galls or Knots.

Galls or knots are known to occur on different parts of plants and are due to several different causes. The one we are considering is not due to any form of insect and is distinct from what is known as the knot of the olive, although its external characteristics are very similar. These galls or knots are more or less spherical, somewhat convoluted outgrowths, and usually of rather soft, spongy tissue, although this may eventually become hard. They usually occur on the stem and main root, just below the surface of the soil. From year to year under favorable conditions they increase in size and may form large excrescences. However, decay usually takes place and the gall rots. This decay may then infect the tree and be the cause of its death. Galls also occur to some extent on the secondary roots, even at some distance from the crown of the tree.

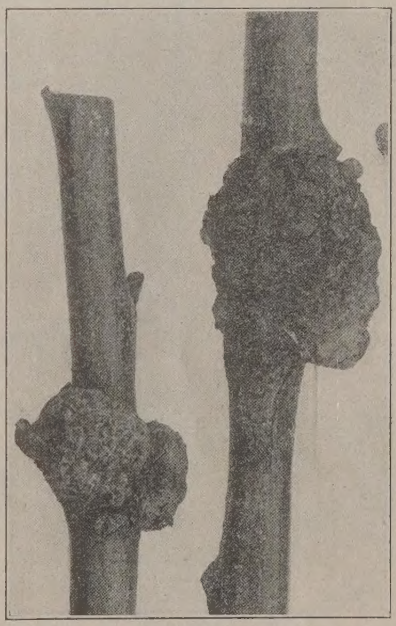

FIG. 2.—Artificial inoculations producing galls on peach, *Prunus persica*. Peach stock suffers badly from crown gall.

### Popular Name.

The disease takes its name from the knots or galls produced and is variably called in literature Root Knot, Crown Gall, Black Knot or Tumors.

### Hosts.

Natural knots have been found on a great variety of plants. The following list, while not supposed to be complete, will at least show the wide range of plants subject to this trouble: Peach, apricot, almond,

prune, plum, apple, pear, quince, English walnut, grape, raspberry, loganberry, cherry, poplar, chestnut, pecan, California black walnut, Eastern black walnut, wild clematis, marguerite or Paris daisy, honeysuckle, *Arbutus unedo*, cotton, alfalfa, beet, willow, hop, red clover, peony.

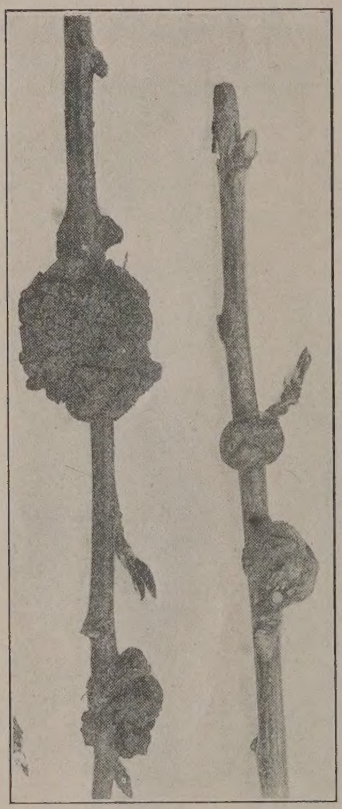

FIG. 3.—Artificial inoculations producing galls on Peen-to hybrid peach.

**Bacterial Nature of Disease.**

There is now abundant proof that these knots are caused by a bacterial organism that enters the tissue through some injury, or possibly at the point where the cotyledons of such seeds as those of the stone fruits are attached to the young plant. In eucalyptus seedlings the natural knots often appear opposite each other where the cotyledons have previously been attached, also the quince knots appear first at the node about the old leaf scar. All the evidence we have goes to show that some injury or weakness is necessary for infection to take place.

**Prevalence of Disease.**

This disease is extremely common in the nurseries of California. Peach rootstock is largely used, as the pits are easily and cheaply secured from the canneries and the root is well adapted to the various stone-fruit trees. The amount of disease varies from year to year, being sometimes as great as 75 per cent. This means a considerable loss to the nurseryman and also to the buyer, if the nurseryman is not careful to cull out affected trees. Buyers of nursery stock cannot be too careful in inspecting their trees before planting, and should rigidly live up to the rule of never planting a tree that shows any signs of small or large knots. There are many almond, peach and prune orchards that today are unprofitable because of this disease. It is impossible to give any estimate in money of the loss due to it but judging from the numerous inquiries and the correspondence on the subject it must be considerable.

## PLAN OF INVESTIGATION.

The importance of this disease in California, and the stimulus imparted from the knowledge of the results being secured by Dr. Erwin F. Smith, led us to attempt to confirm and extend his results under local conditions. Through the courtesy of Mr. George C. Roeding of the Fancher Creek Nurseries we secured many of the rootstocks and pits from which we grew our seedlings, and, upon these, inoculations were made with pure cultures of bacteria which we succeeded in isolating from diseased peach galls.

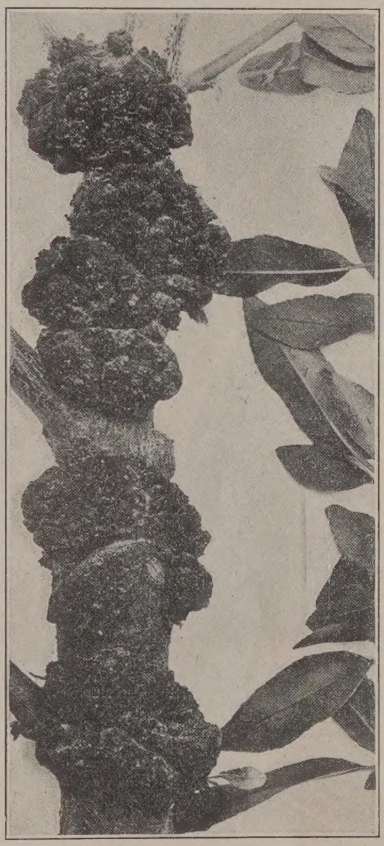

FIG. 4.—Artificial inoculations producing galls on bitter almond. Almond stocks are particularly susceptible to crown gall and the trees are frequently killed by it.

### Isolation of Organism.

In the winter of 1909 an attempt was made to obtain the crown gall organism from peach knot. For this purpose diseased trees were secured from a nursery, and the usual bacteriological methods were employed to secure pure cultures of any organisms that might be present in these galls. From the technical description by Erwin F. Smith of *Bacterium tumefaciens*, we had reason to expect that the pathogenic organism would be characterized by a pearly white growth on nutrient agar. A large number of transfers of such colonies were made from dilution plates and inoculations made in small peach seedlings. Several different cultures were at last secured that produced galls in peach seedlings. One strain of these cultures was used in all the work done in the year 1910. On February 8, 1911, fresh cultures were made from artificial knots produced on *Prunus davidiana* the previous year.

This culture was used for the most part in the series of inoculations carried out during that year.

**Methods of Making Inoculations.**

All inoculations were made from pure cultures isolated from peach root-knots, unless otherwise specified. A culture about forty-eight hours old was used in most of the inoculation work. A steel needle sterilized in a gas flame was used to transfer the growth from the culture tube. In making these inoculations the endeavor was to actually get a considerable amount of the bacterial mass of the culture into the tissue. No attempt was made to produce the disease by applying the germs to the uninjured surface of the plants, and it is very doubtful if this latter method would give anything but negative results.

FIG. 5.—Artificial inoculations producing galls on apricot.

In all cases, unless otherwise indicated, the inoculations were made above ground, on the twigs or trunk of the tree under experimentation. No protection against weather or drying out was made, and few checks were used. These were not considered necessary, as our work was far removed from sources of infection, and in many cases some of the punctures did not take and could be regarded as checks. Our purpose was chiefly to show whether or not these different stocks could be made to produce galls.

It has been stated that cultures of the crown gall organism gradually lose their virulence when grown on artificial media. To test this out, inoculations were made from one culture which had been used in much of the work. This culture, serial No. 649, was isolated December 14, 1909, and sub-cultures made from it from time to time. Inoculations were made from this culture on peach (a Peen-to hybrid) on March 20, 1911, and gave positive results January 25, 1912. A further test was made from culture No. 649 on February 15, 1912. Four branches of the current year's growth were puncture-inoculated on the same peach tree that was used in 1911. June 21, 1912, these showed well-developed galls. In these experiments, cultures over two years old produced well-formed galls.

In some inoculation work on fig, black walnut and pecan, at a time

when the trees were not growing well, knots did not appear until growth again occurred. The organism seemed to be able to live in the tissue for a long period without any apparent formation of galls, but when conditions of growth were again favorable the galls began to develop.

### ARTIFICIAL INOCULATIONS OF PEACH CROWN GALL ON PRUNUS DAVIDIANA. Fig. 1.

This species has never been used as a rootstock in a commercial way, although it has been recommended by the United States Department of Agriculture as a stock for peaches, almond, prunes and plums because of its resistance to drought and alkaline soil. Inoculations were at first made on branches of different sizes of the two varieties *alba* and *rubra*. These trees were growing on the station grounds and in several instances the inoculated branch was bent down and covered with clean river sand. Small seedlings were also inoculated in the glass house.

*P. davidiana rubra* was inoculated on June 21, 1910, on small twigs in the open, using a pure culture of the peach organism. August 5, 1910, indications of the beginnings of small knots were detected. December 8, 1910, observations were again made, and small but definite knots had been produced. Further inoculations on this species were made on July 9, 1910, on twigs one half inch in diameter. December 8, 1910, showed medium sized galls, about one third the size of those on peach (*Prunus persica*). In all this work the galls were smaller than on *P. persica*. February 22, 1912, the tree on which the above experiments had been conducted was dug up and one of the small roots was found to have developed a very large gall. Possibly this infection was due to germs that had been distributed and found their way into the soil. The species does not show any special immunity to this disease.

FIG. 6.—Artificial inoculations producing galls on Myrobolan plum. This is a very popular stock for various stone fruits in California, but it is susceptible to crown gall.

*P. davidiana alba* was also inoculated on the same dates and with the same cultures as was *rubra* and gave fine galls. These seemed to be somewhat more readily produced on *alba* than on *rubra*, but this is without doubt due to a difference in the rapidity of growth of the two trees.

**Summary of results on P. davidiana alba.**

| Date of inoculation. | Result. | Date of observation. |
|---|---|---|
| June 21, 1910 | Positive | December 8, 1910 |
| June 27, 1910 | Positive | December 8, 1910 |
| July 1, 1910 | Positive | December 8, 1910 |
| July 9, 1910 | Positive | December 8, 1910 |
| July 13, 1910 | Negative | December 8, 1910 |
| July 24, 1910 | Positive | December 8, 1910 |
| July 29, 1910 | Positive | December 8, 1910 |
| August 6, 1910 | Positive | December 8, 1910 |

June 27, 1910, a rather large branch was inoculated and immediately covered with clean river sand that was kept moist. This branch remained covered until August 5, 1910, at which time large knots had developed.

FIG. 7.—Artificial inoculations producing galls on mahaleb cherry. Cherries are not so frequently affected in the orchard and nursery as some other stone fruits, but they take the disease readily from artificial inoculation.

Experiments were made with *P. davidiana* seedlings. The pits were obtained from the United States Department of Agriculture by Dr. J. Eliot Coit. They were first sterilized with mercuric bichloride 1 : 1000 for five minutes, rinsed with distilled water and then soaked for three days in water. Pits were cracked and seed planted as follows, on June 24, 1910:

16 seeds in box having sterilized sand.

16 seeds in box having sterilized garden soil.

16 seeds in box having unsterilized garden soil.

25 seeds in box having unsterilized sand.

The soil and sand were sterilized for four hours in autoclave. Boiled water was used for watering sterilized soil. July 14, 1910, inoculated sterilized sand with a forty-eight hour culture of the knot organism growing in Dunham solution. Divided box into two parts, one part in which the seedlings were injured by making punctures with a sterile scalpel just under the surface of the soil, before applying culture of knot organism. There were seven trees in this part of the box. The other half contained ten uninjured seedlings, which were about four inches high at this time. The two check boxes of sand and soil were untreated. December 13, 1910, this experiment was discontinued and showed the following results: In the seven seedlings that were injured there was one tree having a gall. In the ten seedlings that were uninjured there were two trees showing gall. In check boxes of soil and sand there were

thirteen trees and no gall. This experiment is not as conclusive as one would expect, but shows that the disease can be transferred to small *Prunus davidiana* seedlings by inoculating the soil, even if the roots are uninjured.

## ARTIFICIAL INOCULATIONS OF PEACH CROWN GALL ORGANISM ON PEACH (PRUNUS PERSICA). Fig. 2.

The first successful inoculations were made on March 26, 1910, upon small peach seedlings that had been grown in river sand. On May 6th small galls were observed for the first time, appearing on one of the small peach seedlings. This experiment was repeated May 12, 1910, on three small peach seedlings with transfers of the same

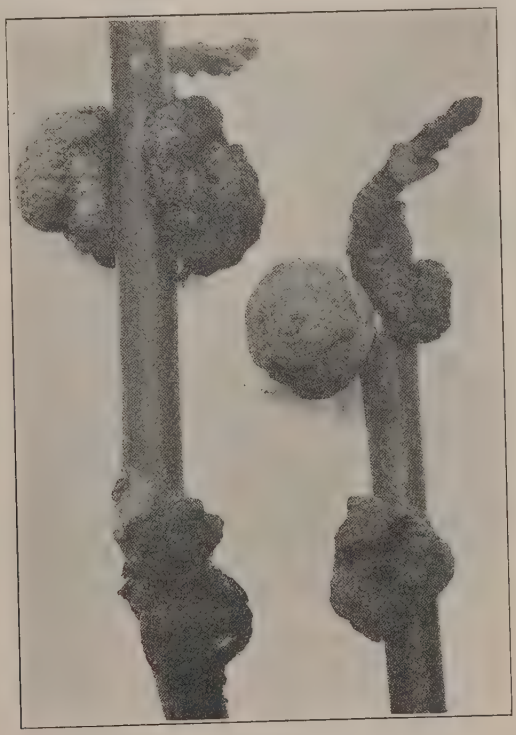

FIG. 8.—Artificial inoculations on English walnut. The various walnut stocks are attacked occasionally in nature, and the disease can be readily induced in any of them by introducing the specific bacterium into actively growing parts.

culture. June 16th small galls were appearing on two of the seedlings; the third tree showed what appeared to be the commencing of knots, but they were not sufficiently definite to be counted. June 21, 1910, small twigs were punctured on a tree in the open without protecting the wounds in any manner. August 5, 1910, fine positive results showed.

SUMMARY OF OTHER SUCCESSFUL INOCULATIONS ON TWIGS OF UNKNOWN VARIETIES OF PRUNUS PERSICA.

June 21, 1910, punctured 2 small twigs. Positive results December 8, 1910.
July 9, 1910, punctured 2 small twigs. Positive results December 8, 1910.
Aug. 6, 1910, punctured 2 small twigs. Positive results December 8, 1910.

All these inoculations were made on branches and were in no wise protected. The knots were very large and well developed.

**Peen-to Peach.**

July 17, 1911, the branches of a Peen-to peach, commonly known as Australian Saucer, were inoculated with pure cultures on the small branches. On October 10, 1911, small galls were to be found. The experiment was discontinued at this time as the tree was cut down.

FIG. 9.—Artificial inoculations in eastern black walnut A, in butternut B.

**Muir Peach.**

July 21, 1911, one small seedling growing in the Station garden was inoculated above ground on the trunk, and on July 31st three more were puncture-inoculated.

January 23, 1912, all these inoculations showed fine positive results, 100 per cent infection.

**Lovell Peach.**

July 31st three Lovell seedlings were puncture-inoculated, followed by three more seedlings on August 3, 1911. January 23, 1912, there were fine positive results in almost every case.

**Salway Peach.**

Inoculations were made upon seedlings as follows:

July 22, 1911, two seedlings inoculated.
July 31, 1911, three seedlings inoculated.
Aug. 3, 1911, two seedlings inoculated.
Aug. 22, 1911, one seedling inoculated.
Sept. 12, 1911, one seedling inoculated.

On January 23, 1912, these experiments were concluded. In every case, except the September 12th inoculation, knots grew where punctures had been made, showing that this variety is susceptible.

**Peen-to (Saucer) Hybrid.** Fig. 3.

This is one of the Florida saucer hybrids that had been grown in a test of varieties. Inoculations were made March 20, 1911, with a year old culture and a more recently isolated culture of the root-knot organism. January 25, 1912, fine large knots were found.

FIG. 10.—Artificial inoculations on southern California black walnut.

FIG. 11.—Artificial inoculations in northern California black walnut.

## ARTIFICIAL INOCULATION OF PEACH CROWN GALL ORGANISM ON ALMOND (PRUNUS AMYGDALUS).

The first experiments were made on the twigs of a large almond of the IXL variety on July 23, 1910. These produced small, hard knots where puncture-inoculated. The tree was not growing very rapidly at this time, which may account for the small size of the knots that developed.

**Hard Shell Almond.**

These seedlings were from pits planted in a nursery row on our Station grounds. They were one to two feet high when inoculated and continued to grow all the fall and most of the winter, the leaves never falling until after new growth commenced in February.

Inoculations were made as follows:

July 15, 1911, two seedlings.
July 22, 1911, two seedlings.
Sept. 15, 1911, two seedlings.

January 23d all the above inoculations showed fine positive results, but the September ones were much smaller than the other galls, which were one to two inches in diameter. These galls were similar to those shown in Fig. 4.

**Bitter Almond.** Fig. 4.

The bitter almonds were fine, vigorous seedlings. They were inoculated on the following dates:

July 22, 1911, one seedling.
July 31, two seedlings.

February 23, 1912, all these inoculations showed fine large knots, one to two inches in diameter.

## ARTIFICIAL INOCULATION OF PEACH CROWN GALL ORGANISM ON APRICOT (PRUNUS ARMENIACA). Fig. 5.

Our first trial of this stock was on the twigs of the cultivated variety Royal. It was inoculated July 23, 1910. September 1st there were small hard knots, about the size of a sweet pea seed. These were observed from time to time during the remainder of the year, but never increased in size. They were trimmed off the following spring.

Apricot pits, variety unknown, were secured from the Fancher Creek Nurseries. These were planted in a nursery row in our Station experimental plot. The seedlings were about one and one half feet high when puncture-inoculated on July 22d and July 31, 1911. January 15, 1912, showed fine large knots on the four seedlings experimented upon. The knots on these apricots are considerably larger than on the peach seedlings, but not so large as those produced upon the hard and bitter almond.

## ARTIFICIAL INOCULATION OF PEACH CROWN GALL ORGANISM ON PLUM. Fig. 6.

Our experiments were confined to four sorts, Myrobolan, Wickson, Marianna and German prune.

**Myrobolan.**

July 3, 1910, puncture-inoculations were made on twigs of a tree about three years old. September 1, 1910, fine large galls had developed. Further experiments were made on seedling stock growing in the Station nursery. June 28, 1911, two small seedlings, and July 17th one seedling, were inoculated. November 1, 1911, these showed galls one to two inches in diameter.

**Marianna.**

Young seedlings growing in the Station nursery were inoculated on small branches. These inoculations were made August 3d and September 15, 1911. February 1, 1912, small galls had developed at every puncture. Lateness in starting the experiment readily accounts for the small size of the knots, and from our limited work we regard this sort of stock as susceptible.

**Wickson Plum.**

The inoculations were on a rather small branch and were made in a slightly different manner from that employed in other cases. Check punctures were first made on one branch. Another branch was then inoculated with a culture of the organism, then both were bent down while still attached to the tree and covered with soil which was kept rather moist. September 16, 1910, showed fine knots on inoculated twig, and none on check branch.

**German Prune** (Italian Prune, Fellenberg).

For two seasons branches of this tree have been unsuccessfully puncture-inoculated with cultures that were used on other hosts with fine positive results. It begins to look as if this particular tree might be resistant to the peach organism, but further

tests must be made to be sure of this. Peach stock has been budded to this prune and the new vigorous growth as well as that on the original tree will be again tested the coming season.

This work may be summarized as follows: July 23, 1910, several puncture inoculations were made on branches of German prune; September 1, 1910, negative results were noted and further observation on December 1st showed no knots. June 28, 1911, July 3, 1911, and July 17, 1911, other puncture-inoculations on the new growth of the tree were made. February 24, 1912, showed no indication of knots and the tissue has in nearly every instance healed, and is nice and smooth at these points.

FIG. 12.—Artificial inoculations on pecan.

## ARTIFICIAL INOCULATION OF PEACH CROWN GALL ORGANISM ON CHERRY STOCK.

**Mazzard.**

These were small trees secured from a nursery and planted out in a row in our Station nursery. This stock seems to be quite difficult to infect.

June 20, 1911, inoculated 1 seedling. January 23, 1912, no galls.
July 17, 1911, inoculated 4 seedlings. January 23, 1912, 4 galls on 1 tree.
July 21, 1911, inoculated 2 seedlings. January 23, 1912, 2 galls on 1 tree.
July 31, 1911, inoculated 1 seedling. January 23, 1912, no galls.
Sept. 15, 1911, inoculated 1 seedling. January 23, 1912, no galls.

**Mahaleb.** Fig. 7.

Positive results on this species are greater in number than on Mazzard and may be summarized as follows:

June 28, 1911, inoculated 1 seedling. January 23, 1912, no galls.
July 12, 1911, inoculated 1 seedling. January 23, 1912, no galls.
July 17, 1911, inoculated 2 seedlings. January 23, 1912, galls on both.

July 21, 1911, inoculated 2 seedlings. January 23, 1912, galls on both.
July 25, 1911, inoculated 2 seedlings. January 23, 1912, galls on both.
July 31, 1911, inoculated 4 seedlings. January 23, 1812, galls on all.

All these successful inoculations showed medium sized galls, but large enough so there is no question as to their nature.

Another experiment was made on August 24, 1910. These inoculations were by puncture on branches of a two-year-old tree. Growth was taking place slowly. July 22, 1911, knots found at four of the points of inoculation.

FIG. 13.—Artificial inoculations on French pear stock. Crown gall is not very rare on pears, but it is not by any means so common or destructive as on the stone fruits. However, the disease is very readily induced by artificial infection of this fruit.

### ARTIFICIAL INOCULATION OF PEACH CROWN GALL ORGANISM ON WALNUT.

**Juglans regia** (English walnut). Fig. 8.

Crown gall of the English walnut has been several times reported in California, and while the disease does not appear to be very widespread, yet in certain localities it is found scattered through the groves. The worst cases are where it occurs just below the surface of the soil. Here the gall growth often seems to rot, probably due to other wood-destroying organisms, leaving the trunk of the tree often more than half eaten away. From observations thus far made, the disease is far more prevalent

on sandy than on other soils. In many places the cutting out practice is employed, the cut surface being painted over with some disinfectant, such as a solution of copper sulphate, or better still Bordeaux mixture. Ranchers state that they sometimes cure the disease by this method.

On July 29, 1910, puncture-inoculations were made on a small English walnut branch. September 2, 1910, inoculations were examined and no gall formation could be discovered. This experiment was regarded as discontinued, but the labels were not removed. July 19, 1911, the writer again visited this tree and was surprised to find fine large galls or tumors.

In the mean time other inoculations had been made on the new growth of an English walnut tree. Inoculated June 3, 1911. November 14, 1911, knots had developed at every puncture. Erwin F. Smith reports having secured positive inoculation of *Juglans regia pendula*, but failed to do so on the straight English walnut.

**Juglans nigra** (eastern black walnut). Fig. 9A.

Crown gall has been before reported as occurring on black walnut. Inoculations were made from the same culture and at the same time as upon the English walnut, July 29, 1910. No results seemed to follow until the following summer when large knots were found, July, 1911. While this tree was planted for a straight Eastern black, it was of California derivation and does not seem to be exactly a typical Eastern black walnut. On June 7, 1912, further inoculations were made on an Eastern black walnut grown from Iowa seed. June 27th showed knots as large as a sweet pea seed.

**Juglans californica** (southern California black walnut). Fig. 10.

Inoculations were made July 23, 1910, by puncture. This experiment for the time was overlooked and no observations made until May 15, 1911. At this time there were fine, large, hard knots at many of the places of inoculation. These inoculations were made on a rapidly growing, two-year-old tree. Another inoculation experiment made July 15, 1910, showed positive results (large galls) December 8, 1910.

**Juglans californica** variety **hindsii** (northern California black walnut). Fig. 11.

In one case we found a root of a two-year-old nursery tree having what appeared to be typical crown gall. The disease rarely occurs on wild California black roots. May 10, 1910, puncture inoculations were made on small Northern California black seedlings that were growing in a pot. August 5, 1910, small knots had appeared at places of puncture. This seems to indicate definite positive results, but further inoculations were made on small seedlings on June 12th and July 29th, 1910. On December 8, 1910, there were small hard galls at points of inoculation.

FIG. 14.—Artificial inoculations on Bartlett pear.

Professor R. E. Smith has observed large crown galls on a Royal hybrid (a hybrid between the Eastern and California black).

**Juglans cinerea** (butternut or white walnut). Fig. 9B.

A small branch was inoculated on July 29, 1910. The gall did not develop to any extent until the following spring, but in July, 1911, small knots were found where punctures had been made. The growth of this tree was slow, as our conditions are not favorable for the rapid growth of this species.

**Juglans sieboldiana** (Japanese walnut).

Inoculations made on July 29, 1910, had produced large galls on July 19, 1911.

## ARTIFICIAL INOCULATION OF PEACH CROWN GALL ORGANISM ON PECAN. Fig. 12.

Inoculations were made on several different dates on seedling pecans growing in the Station nursery. Unsuccessful inoculations were made on July 29, 1910, August 24, 1910, and September 10, 1910. This was due to the slow growth that the trees were making.

August 31, 1911, pecan sprouts were inoculated with pure culture. November 16, 1911, small galls were beginning to appear, but the sprouts were accidentally cut down. September 15, 1911, a branch of pecan was inoculated with pure culture of gall organism. Very small galls were to be observed in the spring of 1912, then followed rapid growth of the tree, and large galls resulted, which were photographed June 18, 1912. When once started in a tree, the organism does not easily lose its virulence.

FIG. 15.—Artificial inoculations producing galls on sour orange. Crown gall is very readily produced on various citrus species by artificially introducing the specific bacteria into actively growing parts, but it occurs very rarely in nature.

FIG. 16.—Artificial inoculations of *B. tumefaciens* on lemon. Aug. 1, 1911-Sept. 30, 1912.

## ARTIFICIAL INOCULATIONS OF POMACEOUS TREES WITH PEACH CROWN GALL ORGANISM.

### French Pear Stock. Fig. 13.

Small seedlings were obtained from a nursery and planted out in row in our Station experimental plot. These seedlings were supposedly imported, as the heading indicates, from France. They are probably of the species *Pyrus communis*. These were inoculated on the rapidly growing branches above ground. The following is the result of work on this stock.

June 28, 1911, inoculated 1 seedling. January 23, 1912, fine large galls.
July 17, 1911, inoculated 2 seedlings. January 23, 1912, fine large galls.
July 21, 1911, inoculated 1 seedling. January 23, 1912, no galls produced.
July 31, 1911, inoculated 1 seedling. January 23, 1912, fine large galls.
Aug. 1, 1911, inoculated 3 seedlings. January 23, 1912, all showed large galls.

Most of these inoculations were positive, many producing large galls, and in some cases these have developed without much apparent growth in length of the inoculated branch. In some cases the galls were partly buried with soil by cultivation.

FIG. 17.—Artificial galls produced on fig. Crown gall has not been found growing naturally on the fig.

FIG. 18.—Artificial galls produced on rose.

### Bartlett Pear. Fig. 14.

Inoculations were made on rapidly growing branches July 9, 1910. September 1, 1910, there were fine large knots at the points of inoculation. Dr. E. F. Smith[1] speaks of the Bartlett as being especially susceptible to the knot, and all the pear stock thus far tested seems to be very favorable for the formation of galls.

### Chinese Wild Pear (Pyrus sp.)

This was from seed secured from the California State Board of Horticulture by Professor E. B. Babcock. Only one seedling was secured and this was planted in the open. The branches were inoculated on August 1, 1911. January 23d the experiment was discontinued. Medium sized knots had developed. The species is probably *P. pashia*.

[1]Crown Gall of Plants, U. S. Department of Agriculture, Bureau of Plant Industry, Bulletin 213, p. 44.

## Apple.

Very little work has been done in experimenting upon apple stock in an endeavor to find a resistant root. The disease does not seem to be as important in this State upon apple as upon the various stone fruits. Mr. Hedgcock,[2] in his exhaustive study of the apple gall, showed that certain varieties have some resistance. Our inoculations, July 29, 1910, were on the White Winter Pearmain, being made in the small branches with pure forty-eight hour cultures of the peach gall organism. The galls developed much more slowly than on most of the hosts experimented upon, but small hard knots were at length produced. Further inoculations were made on August 24, 1910, and these also gave positive results, as small hard galls.

FIG. 19.—Artificial inoculations in pepper tree.

## Angiers Quince.

June 3, 1911, a vigorous-growing sprout of the current year's growth was inoculated. July 17, 1911, further puncture inoculations were made. Up to January 23, 1912, no knots had been found. No further observations were recorded until July 16, 1912, when small knot-like galls from one sixteenth to one eighth of an inch in height were observed to be forming in the healed-up tissue of the old scars of the inoculation of June 3, 1911. This tree also has what is belived to be the regular aerial form of quince knot, described on another page. These are developing at the nodes on the older wood as small prominences, but do not exactly resemble those developing from the artificial inoculations with the peach gall organism. No natural knots have been found on the inoculated branches.

[2]U. S. Department of Agriculture, Bureau of Plant Industry, Bulletin 186.

**Loquat.**

Inoculations have been tried on this host several times and always with negative results, probably due to the slow growth of the tree. Inoculation made September 12, 1911, seemed to show the beginning of small knots, but later observation on June 20th showed the results to be negative.

### INOCULATION OF PEACH CROWN GALL ORGANISM ON CITRUS.

**Valencia Orange.**

Inoculations were made on two small twigs on August 1, 1911. Three knots developed. Two of these were small but the third one was a medium-sized knot on January 23, 1912. These increased in size during the spring months of 1912, owing to the active growth of the tree, and on September 23, 1912, showed well-developed galls.

FIG. 20.—Artificial inoculations producing galls on branch of Victoria bottle tree.

FIG. 21.—Artificially caused galls on forest red gum (*Eucalyptus tereticornis*). Crown gall has not been known to attack the various species of Eucalyptus in nature. The significance of swellings found frequently at the crown of young gum trees is not yet understood. They do not appear to be detrimental to the tree.

**Sweet Lime.**

Inoculations were made at the same time and with the same culture as those in Valencia orange. January 15, 1912, no galls were observed, but March 1, 1912, showed the beginnings of small galls.

**Sour Orange.** Fig. 15.

These artificial inoculations were made on August 1, 1911. The observations of March 1, 1912, showed small, but definite gall formation.

**Lemon.** Fig. 16.

Two twigs of lemons were also inoculated on August 1, 1911, but on March 1, 1912, no galls had developed. On June 21, 1912, medium sized galls had formed.

## ARTIFICIAL INOCULATION OF PEACH CROWN GALL ORGANISM ON MISCELLANEOUS FRUIT-BEARING TREES.

**Fig (Ficas carica).** Fig. 17.

A fig tree was inoculated on two branches August 24, 1910. Observations were taken at various times and no galls developed until early in 1912. At first there were

FIG. 22.—Artificial inoculations on oleander. Similar galls are occasionally found in abundance, occurring naturally.

only slight prominences that could scarcely be taken for galls. The particular branches that were inoculated had not been growing well and, to stimulate new growth, were cut off just beyond where the inoculations had been made. February 22,

1912, one branch showed one fine large knot and several smaller ones. June 18, 1912, this branch was cut off and photographed. The other inoculated branch, on June 15, 1912, showed one small knot. The tree may be growing fast in some parts, but if the branch inoculated is not growing there is little or no gall formation.

**Muscat Grapes.**

July 29, 1910, inoculations were made on two-year-old and present year's growth. September 1, 1910, a small, knot-like growth had developed at each puncture. The vines were not making very rapid growth at this time and because of this the knots produced were small but large enough to indicate positive results.

**Avocado.**

Experiments have been tried on but one tree and that has not been growing very rapidly. These were made in August, 1910, but did not produce galls.

**Olive.**

July 29, 1910, tested out organism on olive shoots. September 1, 1910, negative results. Other puncture-inoculations have been made and these gave negative results. These results agree with those of E. F. Smith.

**Cherimoya (Anona cherimolia).**

Inoculations were made on rapidly growing seedlings on July 31, 1911. February 29, 1912, no knots had been produced.

## INOCULATIONS OF PEACH CROWN GALL ORGANISM ON SOME ORNAMENTAL TREES.

Relatively little is known regarding crown gall on ornamental trees and shrubs, and while the work thus far done is only a beginning in testing out these many different species yet the results secured deserve mention.

**Rose.** Fig. 18.

An unknown variety of climbing rose was inoculated August 24, 1910. February 1, 1911, the experiment was discontinued. Fine large knots had developed.

**Catalina Cherry (Prunus integrifolia).**

These plants were grown from seed and when about six months old were inoculated. Few galls developed, owing to slow growth at time of inoculation.

July 17, 1911, inoculated 2 seedlings. February 23, 1912, 1 knot.
July 22, 1911, inoculated 1 seedling. February 23, 1912, 0 knot.
July 27, 1911, inoculated 1 seedling. February 23, 1912 1 knot.
Aug. 1, 1911, inoculated 1 seedling. February 23, 1912, 0 knot.

**Hill Cherry (Prunus ilicifolia).**

Seedlings of the same age as the Catalina cherries. Inoculations made July 12, 22, 31, 1911. On February 23, 1912, no galls had developed. Too slow growth for knot development.

**Pepper Tree (Schinus molle).** Fig. 19.

This host is exceedingly easy of infection. It should also be noted that even an ordinary puncture with a sterile needle produces a slight, roundish enlargement of the healing tissue, but this is very small as compared with gall development. July 29, 1911, puncture-inoculations were made on a sprout from a large pepper tree, also a branch was punctured with sterile needle as a check. September 1, 1911, medium sized knots had developed from the inoculations. Similar inoculations were made on July 31, 1911, on a small pepper seedling. Large knots had finally developed when the experiment was discontinued on January 23, 1912.

### Victoria Bottle Tree (Sterculia diversifolia). Fig. 20.

A small seedling was inoculated on October 19, 1910, also on August 3, 1911. In both cases knots developed very readily at points of puncture. The small twigs of a small tree were inoculated on September 9, 1911. On March 25, 1912, these inoculations gave definite knots at almost every puncture.

### Flame Tree (Sterculia acerifolia).

A small seedling growing in pot was inoculated on December 12, 1911. Growth of plant slow, but on March 25th the beginnings of small knots were to be observed.

### Forest Red Gum (Eucalyptus tereticornis). Fig. 21.

Seedlings of four to six feet were inoculated. The first successful inoculations were made May 16, 1910. On March 25, 1912, there was one large knot and one very small one at points of inoculation. September 2, 1911, inoculated a seedling about one half inch in diameter. February 20, 1912, there were two small knots. On March 26, 1912, one of these knots had grown rapidly in size, the other had not changed.

Inoculations were made on small seedlings July 29, 1910, on the branches. Typical roundish knots or galls had developed on September 5, 1910.

### Silk Oak (Grevilliea robusta).

Inoculations have been made on the branches of silk oak trees in the open at various times but thus far have only given negative results. This is probably due to the slow growth of the tree.

The several workers of the Bureau of Plant Industry have published their results from inoculating various hosts with the peach gall organism. They secured positive inoculations on the following plants: Daisy, *Pelargonium*, peach, apple, raspberry, rose, sugar beet, and hop. Negative results are recorded on olive, phlox, verbena, grape, *impatiens*, *magnolia*, peony, red oak, Persian walnut and *tradescantia*. It should be stated that unfavorable conditions in many cases would explain many of the negative results above recorded.

FIG. 23.—Knot found on lemon, nearly natural size.

The workers referred to in the above paragraph have also isolated and studied organisms from knots on the following hosts: *Arbutus unedo*, alfalfa, sugar beet, cotton, Paris daisy, grape, honeysuckle, peach, quince, raspberry, rose.

## OTHER GALLS THAT ARE AT PRESENT LITTLE UNDERSTOOD.

### Pecan Gall.

There is, so far as known, no record of crown gall occurring on pecans, but on May 25, 1910, while examining some pecan trees that had been planted on the Whittier Station grounds for about three years, a large gall was found on one of the trees just below the surface of the soil. A portion of the diseased tissue was removed and

cultures made from the material. The appearance of the colonies and growth seemed to be typical of *Bacterium tumefaciens*. Inoculations were made with transfers from these colonies on peach, June 27, 1910. August 6, 1910, showed characteristic gall formation. No further study was made of this strain.

**Lemon Gall.** Figs. 23 and 24.

This gall is not common, but has been found a few times on the branches by Professor H. S. Fawcett, who has figured and described[1] it. The writer is under special obligation to Mr. Fawcett for lemon gall material which he furnished for cultural work and study. Culture from the lemon gall in prune juice gave only negative results, while cultures in standard nutrient agar seem to give characteristic colonies of *Bacterium tumefaciens*. Work is still in progress in their study.

FIG. 24.—A medium sized gall on lemon, reduced.

**Orange Knot.** Figs. 25 and 26.

Some aerial galls were found on a navel orange tree but are of rare occurrence, having been found on but two occasions. Cultures made in prune juice and nutrient glucose agar failed to show any fungous growth that could be regarded as significant. From the work thus far done, it does not seem to be due to *Sphœropsis tumefaciens*.[2] In nutrient agar colonies appeared that closely resemble those of *Bacterium tumefaciens*. At present it is impossible to say what the cause of the knots may be, but work is still being done on this problem.

**Eucalyptus Knot.**

Galls occur frequently on the small seedlings. These are hard knots that occur always at the nodes. There will often be a gall on each side of the plant where

[1]The Monthly Bulletin of State Commission of Horticulture, October, 1912.
[2]U. S. Department of Agriculture, Bureau of Plant Industry, Bulletin 247.

cotyledons or the opposite leaves have previously been. We have had small seedlings with these natural galls under observation for about three years. In some cases there is an increase in size in the knots, which still continue to be hard and do not in any way appear to weaken the trees. In many cases, the diseased trees have com-

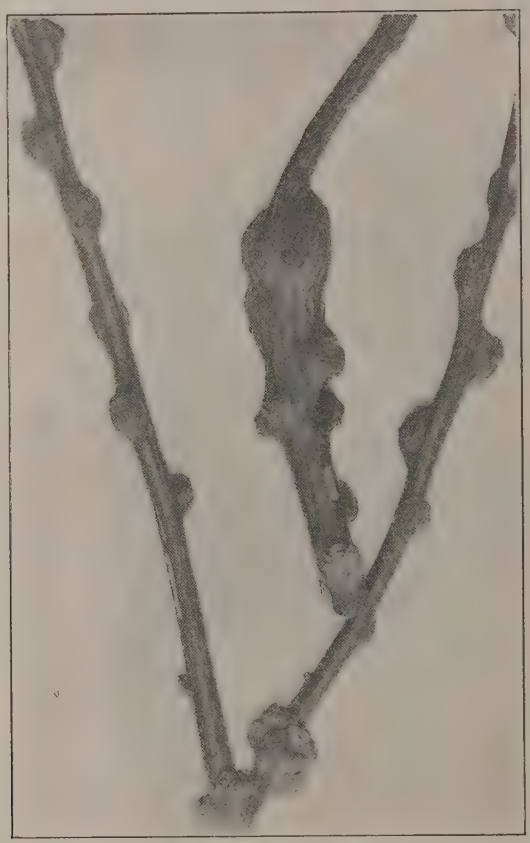

FIG. 25.—Small aerial gall on navel orange branches.

pletely outgrown the disease and in these instances there has been but little increase in size of original knots. From our observations the disease does not seem to seriously injure the growth of the tree. Numerous attempts to isolate a pathogenic organism have resulted in failures, although we still believe the galls may be caused by such an organism.

**Quince.** Figs. 27 and 28.

The very abundant galls on quince branches in California are quite different in appearance from the knots that we have thus far considered, namely those of the apple, peach, pear, and plum. The knots as they are found on young quince trees are small roundish prominences, usually several at a node and near the old leaf scar or at

the base of a branch. In more advanced stages on old twigs the knots are rough or warty outgrowths and always near a node, or at the base of a branch. The effect of these knots on the tree is not often serious, and it is next to impossible to find an old quince tree in California that is free from them. The very commonness of this condition would lead us to question whether it could be caused by a pathogenic organism. Thus far cultures made from the small natural gall on quince have not given a pathogenic organism. Cultures recently made from a large aerial quince gall have produced an organism that is pathogenic on Royal apricot. These inoculations were made May 1, 1912, and good-sized galls were found by July 10, 1912. The galls are round and smooth and do not seem to agree very well with those obtained by Dr. Erwin F. Smith in his work with the quince organism, as he secured rather warty outgrowths.

FIG. 26.—A large gall of orange reduced to about one fourth natural size.

FIG. 27.—Small galls as they occur on quince branches. These represent the early stages of the galls shown in Fig. 28.

## Clematis Gall.

Galls were found on one of our species of wild Clematis at a place where nothing but the wild plant had ever grown. The appearance of the knots seemed to be similar to those caused by the *tumefaciens* organism and could not be confused with nematode or insect galls. Also it should be noted that a recent bulletin* of the Bureau of Plant Industry illustrated natural galls on the branch of mesquite, a wild shrub. It has not definitely been proved that this is due to bacteria but in appearance it resembles those that have been so produced.

*U. S. Department of Agriculture, Bureau of Plant Industry, Bulletin 226, Plate XV.

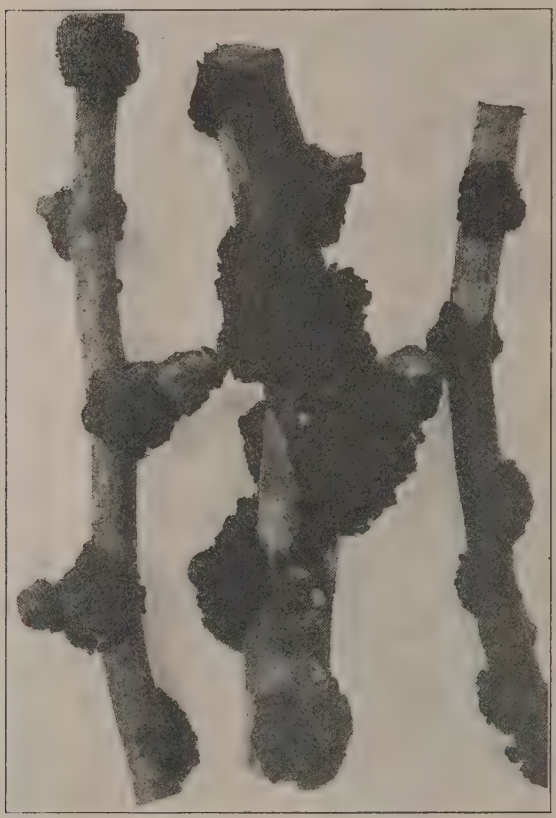

FIG. 28.—Large rough galls on the nodes of quince branches. These galls have not yet been adequately studied.

## SUMMARY AND CONCLUSIONS.

1. *Bacterium tumefaciens* is native to many of the California soils and causes losses in the nurseries that sometimes amount to 70 per cent.

2. Galls can be produced on many different kinds of trees above ground when artificial puncture-inoculations are made with pure culture of the bacterium.

3. The following trees have been successfully infected: several varieties of peach; *Prunus davidiana;* bitter and hard-shell almond; apricot; Myrobolan, Marianna and Wickson plum; Mazzard and Mahaleb cherries; the English walnut; Japanese, Eastern and California black walnuts; pecan; French pear stock; Bartlett and Chinese wild pear; apple; Angiers quince; sour and sweet (Valencia) citrus stock;

sweet limes and lemons; fig; Muscat grapes; eucalyptus; Victoria bottle tree; flame tree; oleander; pepper tree; and *Prunus integrifolia* (Catalina cherry).

4. The following have been puncture-inoculated but have always given negative results: loquat; *Grevilliea robusta* (silk oak); German prune; *Prunus ilicifolia* (wild hill cherry); *Anona cherimolia;* avocado; olive. Some of these are being further tested and may not prove to be resistant.

5. Characteristic aerial galls occur on different kinds of trees in California, the cause of which has not been, in all cases, demonstrated. These knots resemble closely those produced by the crown gall organism.

6. The trees in California thus far observed to have aerial galls are the quince, olive, lemon, sweet orange, oleander and grape.

7. The aerial galls of the olive and grape have been carefully studied and their cause determined. The remainder are being studied, but are at present not well understood.

## STATION PUBLICATIONS AVAILABLE FOR DISTRIBUTION.

### REPORTS.

1896. Report of the Viticultural Work during the seasons 1887–93, with data regarding the Vintages of 1894–95.
1897. Resistant Vines, their Selection, Adaptation, and Grafting. Appendix to Viticultural Report for 1896.
1902. Report of the Agricultural Experiment Station for 1898–1901.
1903. Report of the Agricultural Experiment Station for 1901–03.
1904. Twenty-second Report of the Agricultural Experiment Station for 1903-04.

### BULLETINS.

*Reprint.* Endurance of Drought in Soils of the Arid Regions.
No. 128. Nature, Value, and Utilization of Alkali Lands, and Tolerance of Alkali. (Revised and Reprinted, 1905.)
133. Tolerance of Alkali by Various Cultures.
147. Culture Work of the Sub-stations.
162. Commercial Fertilizers. (Dec. 1, 1904.)
164. Poultry Feeding and Proprietary Foods.
167. Manufacture of Dry Wines in Hot Countries.
168. Observations on Some Vine Diseases in Sonoma County.
169. Tolerance of the Sugar Beet for Alkali.
170. Studies in Grasshopper Control.
171. Commercial Fertilizers. (June 30, 1905.)
174. A New Wine-cooling Machine.
176. Sugar Beets in the San Joaquin Valley.
177. A New Method of Making Dry Red Wine.
178. Mosquito Control.
179. Commercial Fertilizers. (June, 1906.)
181. The Selection of Seed-Wheat.
182. Analyses of Paris Green and Lead Arsenate. Proposed Insecticide Law.
183. The California Tussock-moth.
184. Report of the Plant Pathologist to July 1, 1906.
185. Report of Progress in Cereal Investigations.
186. The Oidium of the Vine.
187. Commercial Fertilizers. (January, 1907.)
188. Lining of Ditches and Reservoirs to Prevent Seepage Losses.
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191. California Peach Blight.
192. Insects Injurious to the Vine in California.
194. Commercial Fertilizers. (Dec., 1907.)
195. The California Grape Root-worm.
197. Grape Culture in California; Improved Methods of Wine-making; Yeast from California Grapes.
No. 198. The Grape Leaf-Hopper.
199. Bovine Tuberculosis.
201. Commercial Fertilizers. (June, 1908.)
202. Commercial Fertilizers. (December, 1908.)
203. Report of the Plant Pathologist to July 1, 1909.)
204. The Dairy Cow's Record and the Stable.
205. Commercial Fertilizers. (December, 1909.)
206. Commercial Fertilizers. (June, 1910.)
207. The Control of the Argentine Ant.
208. The Late Blight of Celery.
209. The Cream Supply.
210. Imperial Valley Settlers' Crop Manual.
211. How to Increase the Yield of Wheat in California.
212. California White Wheats.
213. The Principles of Wine-making.
214. Citrus Fruit Insects.
215. The Housefly in its Relation to Public Health.
216. A Progress Report upon Soil and Climatic Factors Influencing the Composition of Wheat.
217. Honey Plants of California.
218. California Plant Diseases.
219. Report of Live Stock Conditions in Imperial County, California.
220. Fumigation Studies No. 5; Dosage Tables.
221. Commercial Fertilizers. (Oct., 1911.)
222. The Red or Orange Scale.
223. The Black Scale.
224. The Production of the Lima Bean.
225. Tolerance of Eucalyptus for Alkali.
226. The Purple Scale.
227. Grape Vinegar.
228. Pear Thrips and Peach Tree Borer.
229. Hog Cholera and Preventive Serum.
230. Enological Investigations.
231. Walnut Culture in California. Walnut Blight.
232. Commercial Fertilizers.
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234. Red Spiders and Mites of Citrus Trees.

CIRCULARS.

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55. Farmers' Institute and University Extension in Agriculture.
60. Butter Scoring Contest, 1910.
61. University Farm School.
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66. Insecticides and Insect Control.
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69. The Extermination of Morning-Glory.
70. Observation of the Status of Corn Growing in California.
74. Rice.
75. A New Leakage Gauge.
76. Hot Room Callusing.
77. University Farm School.
78. Announcement of Farmers' Short Courses for 1912.
79. List of Insecticide Dealers.
80. Boys' and Girls' Clubs.
81. Sweet Pea Growing Clubs.